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PROCEDURE FOR THE BACTERIOLOGICAL EXAMINATION OF FOOD UTENSILS AND/OR FOOD EQUIPMENT SURFACES

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This Procedure is in conformance with the "Technic for the Bacteriological Examination of Food Utensils, Official Association Procedure," adopted by the Governing Council, American Public Health Association (1948), and the APHA Eleventh Edition, "Standard Methods for the Examination of Dairy Products," 1960.

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PROCEDURE FOR THE BACTERIOLOGICAL EXAMINATION OF FOOD UTENSILS AND/OR FOOD EQUIPMENT SURFACES

Apparatus and Materials - Sterile petri dishes, sterile 1 ml pipettes, Standard Methods agar (tryptone glucose yeast extract agar) sterile forceps or scissors, sterile cotton swabs on standard wooden applicator sticks, and sterile swab containers. Satisfactory containers consist of screw-cap swab bottles 23 X 70 mm or 16 X 100 mm bacteriological test tubes, with cork or rubber stoppers. Other tubes, vials or wide mouth bottles, 1/2 to 1" inside diameter and 2 1/2 to 3 1/2" high with mouth not less than 1/2" in diameter, may be used. Cotton plugs are not satisfactory. The swab holder may be attached to the cap or stopper, or separate dry swabs may be carried in glassine envelopes or sterile containers providing protection against contamination. The swab on the wooden applicator not more than 3" long shall be of firmly twisted nonabsorbent cotton, approximately 3/16" in diameter and 3/4" long. Optionally, metal wire applicators (stiff, not readily corrodible, 1/16" diameter tampered wire) in well-caps may be used provided the swabs are made of nontoxic soluble calcium alginate wool instead of cotton. Soluble wool may also be substituted for nonabsorbent cotton with wooden applicators where it is desired to solubilize the wool instead of shaking thoroughly as described later to disentangle the cotton.

Buffered Distilled Water - The purpose is to secure a sterile solution that is nontoxic to bacteria. Prepare the phosphate buffer solution as follows:

To prepare stock phosphate buffer solution dissolve 34 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water, adjust to pH 7.2 with 1N NaOH solution and make up to 1 liter with distilled water.

Add 1.25 ml stock phosphate buffer solution, 5 ml 10% aqueous sodium thiosulfate (prepared by dissolving $Na_2S_2O_3 \cdot 5H_2O$ at rate of 100 g/liter in distilled water, filter and store stock solution in cool dark place - refrigerator), 4 g Asolectin (Associated Concentrates, Inc., 32-30 61st Avenue, Woodside, Queens, Long Island, N. Y.) and 10 g Tween 20 or Tween 80 (Atlas Powder Co., Wilmington, Del.) to distilled water (freshly distilled water free from toxic contaminants) and make up to 1 liter. When preparing buffered rinse solution containing Asolectin, weigh hygroscopic powder rapidly and dissolve by heating, preferably in double boiler.

Where chlorine and/or quaternary ammonium compounds are known not to be used for sanitizing, and hot water alone has been used as a sanitizing rinse, the neutralizing agents, thiosulfate, Asolectin and Tween may be omitted from rinse solutions.

Distribute the diluted solution in the swab containers in amounts that will provide after sterilizing in the autoclave at 121° C. for not less than 15 minutes, exactly 1 ml for each utensil or surface area of

food equipment to be examined per swab. The containers should be autoclaved either with the swabs in place or in separate containers and the caps should be slightly loosened, during this process. Since the wooden applicator stick and the cap may influence the final pH, spot check the pH of the sterilized buffered distilled water routinely. If necessary, adjust stock solutions before sterilization so that the pH will be between 7.1 and 7.3 after sterilization. If lowering of pH occurs, a more concentrated buffer solution should be used not more than 10 times stronger, i.e., 1 ml of stock phosphate buffer per 80 ml of distilled water.

Sodium Hexametaphosphate Solution - If calcium alginate wool is used, a 10% solution of sodium hexametaphosphate (Calgon) should be prepared by dissolving 10 g Calgon in 100 ml of distilled water and sterilized by autoclaving. When calcium alginate wool is used in preparation of swabs, 0.5 ml of 10% Calgon should be added to each 5 ml of buffered phosphate diluent after the swabs are returned to the laboratory and just before they are to be plated. Alginate wool is soluble in solutions of sodium salts of acids, the calcium salts of which are water soluble.

Collecting Samples - When utensils are to be examined, include at least glasses, cups and spoons, if used, and at least four of each shall be selected at random from the shelves or other places where clean utensils are stored. If a direct check of the dishwashing methods is desired,

utensils should be selected from those recently washed. Care shall be taken to prevent contamination by handling during sampling.

Use 1 swab for each group of 4 or more similar utensils. Take the swab from a freshly opened container of dilution water or dip a sterile swab, if separate, in such dilution water, and squeeze it against the inside of the container so as to remove excess water, leaving the swab moist but not wet. Rub the swab slowly and firmly 3 times over the significant surfaces of 4 or more similar utensils, reversing the direction each time. After swabbing each utensil, return the swab to the container of dilution water, rotate (whip rinse) the swab in the dilution water, and press out the excess water against the inside of the container before swabbing the next of the 4 or more utensils in the group. The significant surfaces of utensils consist of the upper 1/2 in. of the inner and outer rims of cups and glasses and the entire inner and outer surfaces of the bowls of spoons. If it is desired to examine forks and surfaces of dishes, etc., the area to be swabbed should include the entire inner and outer surfaces of the tines of forks, and the inner surfaces of plates and bowls. On plates swab 3 times reversing the direction of each stroke completely across each of two diameters at right angles to each other. On bowls swab 3 times reversing the direction of each stroke around the inner surface at a level at which the swab will hug the surface of the bowl about half way between the bottom of the bowl and the rim.

When swabbing equipment, remove swab from tube of buffered solution (or moisten sterile swab in freshly opened tube of buffered solution), and press out excess liquid on the inside wall of solution container and rub cotton portion of swab slowly and thoroughly over approximately 8 sq. in. of equipment surface. Rub swab slowly and firmly in a path of 1/2 in. width and 16 in. length (to cover approximately 8 sq. in.), rub swab in reverse direction, and repeat initial stroke (so same area is covered by the three strokes). Return swab to original container of buffered solution, rotate (whip rinse) the swab in the diluent and press out excess liquid (leaving swab moist but not wet). Using same applicator, swab four other 8 sq. in. areas of the same piece of equipment (including spots that appear difficult to clean and sanitize), rewetting swab and pressing out excess liquid before swabbing successive areas.

After completion of swabbing five areas (40 sq. in.), replace swab in original container of buffered solution. (If separate dry swab was used, break off wooden applicator stick aseptically on rim of container allowing swab to fall into diluent.) Use a new swab container for each group of utensils, or piece of equipment examined. Keep the container iced while in transit to the laboratory and until it is plated. Plate the diluent water samples preferably within 4 hours of swabbing, but where this cannot be done, samples must be properly refrigerated and analyzed within 24 hours of swabbing.

Laboratory Procedure - If not previously broken, break the wooden swab stick just above the cotton with sterile forceps, or cut aseptically with sterile scissors, or with other sterile cutting device (hot wire glass cutter) allowing the cotton swab to drop into the diluent, where subsequent agitation should thoroughly disentangle the cotton which should be allowed to remain in the container while making transfers. Shake the swab container rapidly, making 50 round trip excursions of 4 to 6 in. with the container in one hand, striking the palm of the other hand at the end of each cycle, and completing the whole in about 10 seconds. Groups of samples may be shaken in test tube holding blocks with similar stroke, speed and abrupt ending of strokes. Shaking machines may be used for time intervals found to disintegrate the cotton swab in a manner equivalent to the prescribed hand method. If metal applicators are used (or if soluble wool swabs are used on wooden applicators so the analyst can reduce the shaking required for completely disentangling cotton), add sterile 10% hexametaphosphate solution to the swab vial at the rate of 0.1 ml for each 1 ml of buffered phosphate diluent. Several to-and-fro hand excursions of the swab vial will aid disintegration and dissolving of the swab from its applicator stick.

Transfer 1 ml of the dilution water to a sterile petri dish. Add approximately 10 ml of melted Standard Methods (tryptone glucose yeast extract) agar, mix, incubate for 48 hours at 32° or 35° C. and count as in making a Standard Plate Count.

In the case of utensils, report the count as the residual bacterial count per utensil surface examined. For example, if four glasses are swabbed, if 1 ml of the 4 ml of the diluent water is plated and if 56 colonies are counted after incubation, record the count as residual bacterial count/glass (RBC/glass) 56.

Interpretation - It is the responsibility of the health officer to fix the standard to be used in the interpretation of the results of this test. However, the Public Health Service recommends a standard of not more than 100 colonies per utensil, or surface area of equipment swabbed. Though this standard does not take into consideration the types of microorganisms present or their public health significance, it does offer a general index of utensil and equipment sanitation. When counts from a particular establishment are frequently or consistently in excess of the standard, improvement of its washing, sanitizing, handling, and/or storage practices is usually indicated.

For equipment on which five areas of approximately 8 sq. in. each have been swabbed, residual bacterial counts not exceeding 500 are satisfactory, i.e., averaging 12.5 colonies per sq. in. of surface or, in the event the five areas swabbed are less than 5 sq. in. each, the counts should not exceed two colonies per sq. cm.

Laboratories not conforming fully with this procedure should have comparative evidence to show that the variations used give comparable results.

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